Attachment B

METHYL BROMIDE

Department of Pesticide Regulation

California Environmental Protection Agency

March 7, 1994

This document is prepared for the Developmental and Reproductive Toxicant Identification Committee for the consideration of methyl bromide as a developmental toxicant under Proposition 65.

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METHYL BROMIDE

I. INTRODUCTION

Methyl bromide is a restricted use pesticide for pest control in structural, soil, and commodity fumigations. In 1991, more than 60 products containing methyl bromide were registered and more than 18 million pounds were used in California (DPR, 1991). Since methyl bromide is acutely toxic at low concentrations, chloropicrin, a lacrimator, is added to some of the methyl bromide formulations as a warning agent.

In 1991, the Methyl Bromide Industry Panel submitted the rabbit developmental toxicity studies conducted by Breslin *et al.* (1990a and 1990b) as required by the California Birth Defect Prevention Act of 1984 (Senate Bill 950). The studies were reviewed by the Medical Toxicology Branch of the Department of Pesticide Regulation (DPR). Significant developmental effects (gall bladder agenesis, fused sternebrae, and decreased fetal body weights) were identified and a No-Observed-Effect Level (NOEL) of 40 ppm was established. This NOEL was used to evaluate the reentry level of 5 ppm established for structural fumigation, and resulted in the conclusion that the reentry level did not provide an adequate margin of safety. In addition, monitoring studies conducted by the DPR's Worker Health and Safety Branch showed that, after structures were cleared for re-occupancy and windows and doors were closed, methyl bromide levels tended to rise above the 5 ppm level. Several days were required for complete dissipation. A preliminary risk assessment was conducted to determine the acceptable level of acute exposure for humans. Using the NOEL from the rabbit developmental study (Breslin *et al.*, 1990b), the acceptable level for acute exposure was calculated to be 210 ppb with a margin of safety of 100. Even though in the developmental study the exposure was continuous during a selected period during gestation (e.g. from gestation days 7 to 19), the assumption is that only a single exposure at a critical time is necessary for the induction of developmental adverse effects according to the U.S. Environmental Protection Agency (USEPA) Guidelines for Developmental Toxicity Risk Assessment (USEPA, 1991).

DPR promulgated emergency regulations which required a longer aeration period after fumigation, and required pest control operators to hand out a fact sheet explaining the potential hazards of methyl bromide fumigation. The fact sheet was prepared by DPR in consultation with the Office of Environmental Health Hazard Assessment (OEHHA), California Department of Health Services, and USEPA. A summary of the animal toxicology was included in the fact sheet describing the effects to the central nervous system, eyes, respiratory system, and fetal development. The section on fetal development is reproduced below:

> "In recent animal studies, methyl bromide caused birth defects when pregnant animals were exposed under experimental conditions. There is no evidence that methyl bromide affects human reproduction, although some chemicals which cause birth defects in animals may also cause birth defects in humans. Any person, including pregnant women, should avoid unnecessary exposure."

A similar warning for developmental effects was subsequently required by USEPA on methyl bromide product labels used for structural fumigation. On January 1, 1993, methyl bromide was administratively listed as a developmental toxicant by OEHHA under Proposition

65. The Proposition 65 statute requires the listing of a chemical when the state or federal government has formally required the labeling or identification of the chemical as a carcinogen or a reproductive toxicant. Warning would have been required for all uses of methyl bromide as of January 1, 1994.

An administrative petition from the growers and users of methyl bromide to rescind the listing of methyl bromide was submitted and subsequently denied by OEHHA in October, 1993. A Safe Use Determination (SUD) was requested by the Alliance of the Methyl Bromide Industry. A workshop on the SUD was conducted on November 30, December 1, and a public hearing was held on December 20, 1993. On December 21, 1993, OEHHA modified the listing from "methyl bromide" to "methyl bromide as a structural fumigant". The broader question of whether methyl bromide should be listed for all uses was to be referred to the Developmental and Reproductive Toxicant Identification Committee of the OEHHA Science Advisory Board.

II. TOXICOLOGY PROFILE

The following is a brief discussion of the pharmacokinetic and toxicity studies of methyl bromide in humans and in laboratory animals. Pharmacokinetic studies with ^{14}C-methyl bromide showed that methyl bromide equivalents are distributed to all tissues in the body. Acute, subchronic, and chronic studies showed toxic effects in multiple organs. After methyl bromide inhalation exposure, neurotoxicity has been reported in humans, rats, mice, rabbits, guinea pigs, and monkeys. Specific studies which showed developmental and reproductive effects are presented in section **III. DEVELOPMENTAL AND REPRODUCTIVE TOXICITY**. Additionally, genotoxicity studies which showed that methyl bromide is a direct acting mutagen and that it can alkylate DNA and proteins are also presented in this section.

A. Pharmacokinetic studies

After inhalation, intraperitoneal, and oral routes of administrations, methyl bromide (^{14}C-methyl bromide) is rapidly absorbed and radioactivity is detected in all tissues (Medinsky *et al.*, 1985; Bond *et al.*, 1985; Jaskot *et al.*, 1988; Raabe, 1986, Raabe, 1988; Medinsky *et al.*, 1984). After inhalation exposure, the percentages of administered doses absorbed were similar in several species; 48% in the rat, 40% in the dog, and 51-55% in human. In the rat, the highest levels in the tissues, principally in the lungs, were reached immediately after exposure. Following oral or intraperitoneal administration, the highest levels of radioactivity were found in the liver and kidneys of the rat. Methyl bromide was extensively biotransformed into unidentified products and carbon dioxide. In the rat within 1 hour after exposure, less than 10% of the radioactivity in the tissues was intact methyl bromide. Carbon dioxide accounted for almost 50% (inhalation and intraperitoneal routes), and 30% (oral route) of the radioactivity excreted in the exhaled air. After inhalation exposure, the percent of absorbed doses excreted in the air and urine were much lower in the human study than those found in the rat study. The difference may be due to the time when samples were taken. In the human study, the samples were taken after only 0.5 hour of clearance and not at steady state. The primary routes of excretion were the exhaled air for inhalation and intraperitoneal exposures, and the urine for the oral route of administration. After oral administration, there was reabsorption of biliary metabolites of methyl bromide from the gut.

B. Toxicity Studies

1. Humans

The primary route of exposure to methyl bromide by the general population and workers is via inhalation. In California, there were 148 illness associated with methyl bromide reported in 1982 through 1990 (Worker and Health Safety Branch, 1993). In the work place, systemic illnesses in the workers are generally due to equipment failure or other accidents. For the general population, exposure is due to drift or leakage of methyl bromide from fumigated fields, chambers, or homes; or due to residual levels of methyl bromide in fumigated homes. The general population also may be exposed to methyl bromide by dietary exposure from ingestion of fumigated commodities. However, the residue levels are relatively low or below the analytical detection limit.

Signs and symptoms in humans from inhalation exposure to methyl bromide are dependent on concentration and exposure duration (von Oettingen, 1946, Rathus and Landy, 1961; Greenberg, 1971; Grant, 1974; Anger *et al.*, 1986; Gehring *et al.*, 1991). Acute exposure to lethal concentrations results in early symptoms of malaise, headache, visual disturbances, nausea, and vomiting. Later symptoms include delirium, convulsions, and respiratory failure or cardiovascular collapse leading to death. Nonlethal exposures result in neurological effects similar to the early symptoms for fatal exposure. Symptoms may persist after exposure, depending on the severity of the toxicity. Dermal exposure of workers to concentrated methyl bromide as a liquid results in vesication and swelling of the skin (Butler *et al.*, 1945; Jordi, 1953; Zwaveling *et al.*, 1987).

Recently, Hallier *et al.* (1993) showed that there is a polymorphism in human blood for glutathione-S-transferase activity directed at methyl bromide. Of the individuals studied, 75% of them are considered conjugators; that is, there was an apparent enzyme-mediated disappearance of methyl bromide when their erythrocyte cytoplasm was incubated with methyl bromide and glutathione. Individuals whose blood did not show such a reaction are called non-conjugators. The conjugation reaction is apparently a detoxification mechanism because the levels of sister chromatid exchanges in the peripheral lymphocytes of non-conjugators were increased by approximately two-fold over untreated control levels when their whole blood was incubated in the presence of methyl bromide. Under identical testing conditions, lymphocytes from conjugators showed little or no increase in their levels of sister chromatid exchanges.

2. Laboratory Animals

a. Acute Toxicity

Methyl bromide is a Toxicity Category I compound because of its acute inhalation toxicity (Federal Register, 1991). The lethal air concentrations for 100% death (LC100) are 220 ppm for rats after 26 hours (Irish *et al.*, 1940), 220 ppm for rabbits after 32 hours (Irish *et al.*, 1940) and 490 ppm for guinea pigs after 8 hours (Sayers *et al.*, 1929), compared to 1583 ppm for humans after 10-20 hours (USEPA, 1986).

The nonlethal effects in laboratory animals exposed acutely to methyl bromide involve multiple organs. These effects include alteration in glutathione transferase activity,

catecholamine levels, and tyrosine hydroxylase activity (Jaskot *et al.*, 1988; Davenport *et al.*, 1992; Honma *et al.*, 1987, Honma *et al.*, 1991). Tissue damage has been found in the lungs, nasal cavity, brain, kidneys, testes, and adrenal glands (Irish *et al.*, 1940; Hurtt *et al.*, 1987; Hurtt *et al.*, 1988; Hurtt and Working, 1988; Hastings, 1990; Sayers *et al.*, 1929). Clinical signs of neurotoxicity include ataxia, paralysis, tremor, and convulsion (Hurtt *et al.*, 1987; Irish *et al.*, 1940; Alexeeff and Kilgore, 1983 and 1985; Sayers *et al.*, 1929). Signs of acute oral toxicity include prostration, increased heart rate, lesions in multiple organs including the stomach and brain, hypoactivity, hypothermia, and death (Naas, 1990).

b. Subchronic Toxicity

Subchronic inhalation exposure of laboratory animals to methyl bromide also results in neurotoxicity, tissue degeneration (such as brain, nasal cavity, heart, adrenal glands, thymus, spleen, kidneys, and testes), and death (Irish *et al.*, 1940; NTP, 1992; Eustis, 1992; Eustis *et al.*, 1988; Kato *et al.*, 1986; Drew, 1983; Anger *et al.*, 1981). Based on overt signs of neurotoxicity, the rabbit was more sensitive than other species to methyl bromide; and the sensitivity in decreasing order is rabbit > monkey > guinea pig > rat (Irish *et al.*, 1940). For subchronic oral exposure, the primary finding was hyperplasia in the forestomach (Danse *et al.*, 1984; Boorman *et al.*, 1986; Hubbs, 1986).

c. Chronic Toxicity and Oncogenicity

The nasal cavity, brain, and heart were also target organs in rodents after chronic inhalation exposure to methyl bromide. Olfactory epithelial damage (hyperplasia, metaplasia, and necrosis) and myocardial degeneration were observed in rats and mice (Reuzel *et al.*, 1987 and 1991; NTP, 1992; Eustis, 1992). Cerebellar and cerebral degeneration was detected in mice (NTP, 1992; Eustis, 1992).

3. Genotoxicity Studies

Methyl bromide was mutagenic in in vitro mutation assays and was a base-pair substitution mutagen in the Salmonella assays (Simmon *et al.*, 1977; Kramers *et al.*, 1985; Moriya *et al.*, 1983; NTP, 1992; Eustis, 1992). It is considered a direct acting mutagen since liver S-9 fraction was not required for mutagenicity (Kramers *et al.*, 1985; NTP, 1992; Eustis, 1992). There was micronuclei formation in the female mice (NTP, 1992 and Eustis, 1992) and increased frequency of sister chromatid exchanges in CHO cells, mouse bone marrow cells in vivo, and lymphocytes (Rounds, 1980; NTP, 1992; Eustis, 1992; Garry *et al.*, 1990). Methyl bromide did not induce unscheduled DNA synthesis in rat hepatocytes, or cause sperm abnormalities in mice (Kramers *et al.*, 1985; McGregor, 1981).

Methyl bromide is a direct-acting alkylating agent. In vivo exposure of rats and mice to methyl bromide resulted in the alkylation of tissue DNA. After exposure to methyl bromide by either inhalation or intraperitoneal routes, 7-methylguanine in liver and spleen DNA, as well as methylated cysteine in hemoglobin and liver protein, were detected (Djalali-Behzad, *et al.*, 1981). In a study by Gansewendt, *et al.* (1991), 7-methyl guanine and O^6-methyl guanine were detected in the DNA from the liver, lung, stomach, and forestomach of rats exposed to methyl bromide by inhalation or by gavage. The formation of DNA adducts indicated a systemic genotoxic effect since the highest concentrations of the DNA adducts were found in the

stomach and forestomach DNA for both routes.

C. Structure-Activity Relationship

Methyl chloride, the chloro analog of methyl bromide, is a known developmental toxicant. Heart defects were found in fetuses of pregnant mice exposed by inhalation to methyl chloride during gestation (Wolkowski-Tyl *et al.* 1983a). The significance of the finding was further discussed (John-Greene *et al.* 1985 and Tyl, 1985). Exposure of pregnant rats to the same methyl chloride concentration did not result in any developmental effects (Wolkowski-Tyl *et al.*, 1983b). Further studies with mice and rats showed a reduction of nonprotein sulfhydryl concentrations in fetal tissues, which suggested transplacental passage of methyl chloride and/or its metabolites (observations discussed in Wolkowski-Tyl *et al.*, 1983b)

III. DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

In this section, studies which showed developmental and reproductive toxicological effects are discussed and a summary is presented in Table 1. Work sheets for each of the studies are available from DPR. In the evaluation of the data and the determination of whether regulatory action to decrease methyl bromide exposure was appropriate, DPR used the basic assumptions in the USEPA Guidelines for Developmental Toxicity (USEPA, 1991) and Kimmel *et al.* (1993), and they are:

1. An agent that produces an adverse developmental effect in experimental animal studies will potentially pose a hazard to humans following sufficient exposure during development.

2. All of the four manifestations of developmental toxicity (death, structural abnormalities, growth alterations, and functional deficits) are of concern.

3. The types of developmental effects seen in animal studies are not necessarily the same as those that may be produced in humans.

4. The most appropriate species is used to estimate human risk when data are available. In the absence of data, it is assumed that the most sensitive species is appropriate for use, based on observations that humans are as sensitive or more so than the most sensitive animal species tested for the majority of agents known to cause human developmental toxicity.

5. In general, a threshold is assumed for the dose-response curve for agents that produce developmental toxicity.

A. Rats

1. American Biogenics Corp., 1986

In a study conducted by American Biogenics Corp. (1986), parental rats were exposed to methyl bromide 6 hrs/day, 5 days/week at 0, 3, 30, or 90 ppm at premating, mating, gestation, and lactation, except from gestation day 21 to lactation day 4. There was a total of 4

matings, generating F1a and F1b offspring from the F0 parents and F2a and F2b offspring from the F1 parents. The first and second generation pups were exposed to methyl bromide only in utero for 5 days per week from gestation days 0 to 20, resulting in a total exposure of 15 days. Offspring were not exposed to methyl bromide during the lactation period. The F1 parents were selected from the F1b offspring. The F1 animals were then exposed to methyl bromide after weaning. The offspring from the F1 parents are called the F2a and F2b litters.

Absolute brain weights were decreased in the 90 ppm F0 males, F1 males, and F1 females, and possibly in the 30 ppm F1 females as well. In the second mating of the F1 adults, the fertility index decreased from 90.9% in the controls to ? 68% in the 30 and 90 ppm groups.

The results on pup body weights are shown in Table 3. At birth, the F1a and F1b pup body weights for the treated groups were either higher or not significantly different from controls. However, the pups from the 30 and 90 ppm groups in the F1a and F1b showed significantly reduced body weights on lactation days 14 to 28. The F2a pup body weights of the 90 ppm group were lower at birth than the controls and remained reduced throughout lactation. The F2a pup body weights of the 30 ppm group showed significant reduction on lactation days 14 to 28. The F2b litter body weights for the 30 and 90 ppm groups were decreased, starting as early as 4 days after birth. The reductions in body weight tended to be greater in the F2a and F2b progeny (reduction of 9 to 21% at 90 ppm) compared respectively to the F1a and F1b pups (reduction of 5 to 11% at 90 ppm). Since the pups were not exposed to methyl bromide during the lactation period, except perhaps via the maternal milk, the finding of reduced body weights suggests that growth retardation may be an effect due to in utero exposure. Except for a reduction of food consumption in the first week of exposure of the parents of the first generation, the food consumption of all the parents were comparable to the control animals. No change in nursing behavior was reported. Pending the submission of additional information on exposure conditions and histological examinations of target organs, the tentative NOEL is 3 ppm for decreased body weights in the neonates, and decreased fertility in the dams.

2. Sikov *et al.*, 1981

In the developmental study by Sikov *et al.* (1981), nonpregnant Wistar rats were exposed to methyl bromide concentrations of 0 (air), 20, or 70 ppm for 7 hours/day for 3 weeks. These rats were then allowed to mate with untreated males. Upon evidence of mating, females were then assigned to gestational exposure groups in the following manner. Females exposed to 0 ppm methyl bromide pregestationally were proportioned into groups that would be exposed to 0, 20, or 70 ppm methyl bromide during their gestational periods. Females exposed pregestationally to 20 or 70 ppm methyl bromide were proportioned into groups that would be exposed to 0 ppm or to the same methyl bromide concentration that was used pregestationally. Gestational exposures were for 7 hours/day and were continuous from gestation days 1 through 19.

There was no maternal toxicity at any of the doses. The only developmental effect identified by DPR was an increase in the incidences of delayed skull ossification of the supraoccipital plate in the fetuses of both groups exposed to 70 ppm during gestation (Table 2), an effect considered a skeletal variation. The authors of the report had noted an increase in the incidences of delayed ossification for several skeletal structures, but these were considered not to be treatment-related. However, since the skull effect was noted in both groups exposed to the high dose (70 ppm) during gestation, DPR did not consider the dismissal appropriate as a NOEL can be established at 20 ppm. It has also been suggested that the skeletal results in this

study were highly variable and, therefore, no treatment-induced effect can be inferred. DPR evaluated all of the skeletal results and only identified the skull ossification defect as a treatment-induced effect. Other skeletal endpoints were not considered treatment-related because there was no indication of an increased incidence of the effects in the treated groups (e.g., rudimentary rib), or the increased incidence was seen only in one of the 70 ppm groups, but not the other 70 ppm group (e.g. unossified sternebrae).

It has been suggested that since there were 5 treated groups in this study, the likelihood of a statistically significant effect by chance was proportionately increased. DPR does not believe that the increased incidence of skull ossification defects was due to chance since the effect was observed at the same litter incidence in both 70 ppm groups (Table 2).

That the delayed ossification effect in this study is only a skeletal variation and that other skeletal effects were not noted is acknowledged. However, this effect appears to be treatment-induced and was seen in the absence of any maternal toxicity. That is, the highest dose tested, 70 ppm, was not a maximum tolerated dose. Results from Eustis *et al.* (1988) suggested that the maximum tolerated dose would be between 90 and 160 ppm. Results from the rat reproduction study showed no parental toxicity in rats exposed to methyl bromide at 90 ppm for two matings per generation for two generations for a total of 132-145 days of exposure. Therefore, if the methyl bromide concentration in the Sikov *et al.* study were higher, then more severe developmental effects might be expected.

B. Rabbits

1. Sikov *et al.*, 1981

Sikov *et al.* (1981) also conducted a study with rabbits. Pregnant New Zealand white rabbits were to be exposed to methyl bromide concentrations of 0, 20, or 70 ppm, 7 hours/day, on gestation days 1 to 24. Neurotoxicity (convulsion and paresis in the hind limb) was observed in the 70 ppm group after 1 week of exposure and deaths started to occur starting on gestation day 9. Because of neurotoxicity and mortalities in the 70 ppm group, exposures of all the groups were stopped on gestation day 15 (Hardin *et al.*, 1981). By gestation day 30, all but one of the 70 ppm does were dead. No developmental effects were observed in the fetuses of the 20 ppm does or those of the one survivor from the 70 ppm group. Because of the loss of the 70 ppm group and the abbreviated duration of gestational exposure in the 20 ppm group, this study is not a valid developmental study and should not be interpreted as "negative" evidence for developmental toxicity. This study does serve to illustrate that pregnant rabbits are significantly more susceptible than pregnant rats to the neurotoxic effects of methyl bromide. That is, neurotoxicity and death occurred within 9 days of continuous exposure of rabbits to 70 ppm in the Sikov *et al.* study whereas no maternal toxicity was observed in rats exposed to methyl bromide at the same concentration pregestationally as well as from gestation days 1 to 19.

2. Breslin *et al.*, 1990a

In a probe study conducted by Breslin *et al.* (1990a) to determine the maternal toxicity and embryolethality of methyl bromide, pregnant New Zealand white rabbits were exposed to methyl bromide 6 hours/day on gestation days 7 to 19, with sacrifice on gestation day 20. There were two parts to this study. In Part I, the methyl bromide concentrations were 0, 10, 30, or 50 ppm. No maternal or fetal effects were observed. In Part II, the methyl bromide concentrations were 0, 50, 70, or 140 ppm. All does in the 140 ppm group showed signs of

toxicity (lethargy and decreased food consumption) after 8 exposures. With continued exposure, severe neurotoxicity was observed, including the following: lethargy, labored breathing, ataxia, right-sided head tilt, reduced sensation in the extremities, dilated pupils, lateral recumbency, loss of placing or righting reflex, and rear leg splay. As a result, all rabbits in this group were sacrificed on gestation day 17. Histological examination was done on brains from all treatment groups. Only brains from the 140 ppm group showed pathological lesions. Fetal examinations were limited to counting the number of implantations and resorptions. In the 70 ppm group, the only possible effect was a reduction in litter size that was associated with an increased incidence of preimplantation loss. However, this was not considered to be treatment-related because treatment-related preimplantation loss would not be expected with this study design, since treatments did not start until after implantation had occurred. Also, an increase in preimplantation loss was not seen in either of the 80 ppm groups that were part of the definitive study (Breslin *et al.*, 1990b).

3. Breslin *et al.*, 1990b

In the definitive study by the same group of investigators, there were also two parts to the study (Breslin *et al.*, 1990b). In the first part (Part I), pregnant rabbits were exposed to methyl bromide concentrations of 0, 20, 40, or 80 ppm for 6 hours/day on gestation days 7 to 19 and were sacrificed on gestation day 28. The second part (Part II) was designed to determine if the gall bladder agenesis observed in Part I was associated with a particular male that was one of several males whose sperm were mixed together and used for artificial insemination. The methyl bromide concentrations were 0 or 80 ppm.

Maternal effects were observed in the 80 ppm group. In Part I, signs of neurotoxicity (right-sided head tilt, ataxia, slight lateral recumbency, lethargy) were observed in 3 of 26 does in the 80 ppm group. The severity of these neurological effects in these three affected does was much less than that seen in the 140 ppm does in the probe study. Also, the onset of these signs was later (Table 1). In Part II, none of the 17 does in the 80 ppm group exhibited signs of neurotoxicity.

Decreased body weight gain was observed in the does across treatment groups in a variable fashion. The body weight gain for gestation days 7 to 20 was reduced in both 80 ppm groups but only the reduction in Part II was statistically significant. This reduction in maternal body weight gain in the 80 ppm group in Part II was seen in the presence of reduced fetal weight. The biological significance of the maternal body weight gain reduction is uncertain because body weight changes in rabbits during pregnancy are more variable than other species (USEPA, 1991).

The incidences of fetal effects considered by DPR are shown in Table 4. The effects observed included omphalocele, retroesophageal right subclavian artery, hemorrhage with or without generalized edema, gall bladder agenesis, fused sternebrae, and decreased fetal body weight.

Omphalocele and retroesophageal right subclavian artery were seen only in the 80 ppm group in Part I whereas hemorrhage (with or without generalized edema) was seen in both 80 ppm groups. Although their incidences were low and not statistically different from the incidences in the concurrent controls, these effects were considered to be possibly treatment-related because each of these effects has been rarely seen at the Dow laboratory in its control groups (Table 5). The Dow laboratory database, from 1974 to 1991, did not record a control group with two affected litters for any of these three endpoints (omphalocele, retroesophageal

right subclavian artery, and hemorrhage, with or without generalized edema) nor a control group wherein these three findings were made in the same group. Data in the database compiled by the Middle Atlantic Reproduction and Teratology Association (MARTA, Lang 1993) also corroborated that these are rare findings in control groups (Table 7).

The incidences of gall bladder agenesis and fused sternebrae were significantly different from the controls at $p \leq 0.05$ level. These effects were considered independent of maternal toxicity because these effects were observed in fetuses from both normally behaving and affected (with neurotoxicity) does. The finding of gall bladder agenesis was confirmed in Part II of the experiment with approximately the same litter incidence (29%) as for Part I (26%). No maternal toxicity was reported in Part II. The possibility that gall bladder agenesis was associated with a particular male was discounted since the malformation was not observed in the naive controls (in Part II) which had been inseminated with the sperm from a suspect male.

It has been suggested that the finding of gall bladder agenesis is biologically insignificant. It has been noted that some species, like the rat, do not have a gall bladder. DPR considers the absence of the gall bladder a significant biological finding. First, humans do have gall bladders. Second, gall bladder agenesis is a malformation; that is, the entire organ did not form. Histological investigation done on one case of gall bladder agenesis from the 80 ppm group (Part I) confirmed the failure of the gall bladder to form, including the absence of the common bile duct. As stated earlier, it is assumed when using animal data for identification of human hazards, that a chemical will not necessarily cause the same defect in different species. If a chemical is shown to cause a malformation in one species, it is assumed, unless proven otherwise, that the chemical has the potential for causing a malformation (not necessarily the same malformation) in the human population (USEPA, 1991; Kimmel *et al.*, 1993).

It has been suggested that since gall bladders vary in size and shape, the lack of a gall bladder is only part of a continuum of effects; therefore, gall bladder agenesis should be considered a variation. DPR does not concur with this conclusion in that the results from the Breslin study clearly identified the absence of gall bladder, not a reduction in size or change in shape. For the same reason, the fact that the spontaneous incidence of hypoplastic gall bladders may be considerably greater and more variable than that for gall bladder agenesis is considered inconsequential to interpreting the results of the Breslin *et al.* study.

It has been suggested that developmental effects should be compared with historical control databases because the evaluation of these effects are subjective and variable. Because of the variability, DPR believes the use of in-house historical control data (Dow Chemical Company as the contract laboratory for the study) is most appropriate, especially those data generated closest in time to the study in question. The in-house historical control data would be more relevant than historical control data generated outside of the facility in question. DPR specifically considered the historical control data from the contracting laboratory of the Breslin *et al.* study. With regard to gall bladder agenesis, the facility did not record a single case in its control groups between 1974 and 1989 when the methyl bromide study was conducted. However during this time, the facility did identify hypoplastic gall bladder and gall bladder agenesis as possible treatment-induced effects in studies of other chemicals. The overall litter incidence for the control groups is 0.35% for rabbits used at the facility between 1974 and 1991 (i.e., including two years after the methyl bromide study was conducted; Table 5). This incidence rate is comparable to the 0.67% (7 affected litters/1051 litters) calculated for the absence of gall bladder in the negative control groups for 37 studies performed between 1985 and 1993 at the WIL Research Laboratories (Holson, 1993a, 1993b, and 1993c, Table 6). In addition, DPR examined the historical control databases compiled by MARTA (Table 7,

Lang, 1993) and those published by Stadler *et al.* (1983) (Table 8). These databases showed that the spontaneous litter incidence for gall bladder agenesis is less than one percent. They supported the DPR conclusion that the finding of ? 26% litter incidence for gall bladder agenesis in the Breslin *et al.* study is significant.

There was a dose-related increase in the incidences of fused sternebrae in the fetuses of the methyl bromide treated groups. While DPR considered this a significant finding, others have questioned it because the Breslin *et al.* study facility's historical control database contained an entry for "unfused sternebrae", a term that could be considered unconventional. Therefore, a question was raised regarding what was considered to be "fused" sternebrae. DPR examined the historical control database supporting the Breslin *et al.* study. A total of only 22 fetuses (involving 21 litters) had this entry. These "unfused" sternebrae entries involved studies conducted between 1974 (earliest study in the database) and 1982, whereas the Breslin *et al.* study was not conducted until 1989. Dr. Breslin was contacted to determine the definition of "unfused" sternebrae. He indicated that this term referred to a type of delayed ossification wherein the sternebrae present as two parts separated longitudinally; it is also called a bipartite sternebrae (Figure 1). The reason that there are no findings of unfused sternebrae after 1982 is because it was decided in that time period to simply include this finding in the general tally of sternebrae exhibiting delayed ossification. The reason the database still has unfused sternebrae as an entry is to maintain its completeness and authenticity as a historical record. The litter incidence for fused sternebrae was 53% for the 80 ppm group. The litter incidence rates in the historical control databases are 4.46% for the Breslin *et al.* study facility (Table 5), 1.58% for the WIL Research Laboratories (Table 6), and 4.58% for the MARTA database (Table 7). Therefore, the DPR concludes that there is no basis to discount the finding of an increased incidence of fused sternebrae in this study. Also, the fact that skeletal examinations were not conducted in Part II of the study has no consequence on how the skeletal findings of Part I are interpreted.

In Part II of the experiment, at 80 ppm, there was also a significant decrease in the fetal body weight. This decrease in fetal body weight also resulted in a significant decrease (79% of control values) in gravid uterine weight (the total weight of the uterus and fetuses) of does in this group. It has been suggested that this effect should be discounted since the decrease was statistically significant only in Part II and not Part I. Also, when the Part II-80 ppm fetal body weight data are compared to the Part I control (0 ppm) data, there is no significant difference between the two values. This conclusion is not valid since the proper control group for Part II for the 80 ppm group is its concurrent group. It is not known why fetal body weights were affected significantly in Part II and not in Part I, but it may be due to the following differences between Parts I and II: (1) animals in Part II were from a different shipment of rabbits (albeit from the same supplier); (2) animals in Part II were 2-3 months younger than used in Part I; in fact, their body weights (3.3-3.4 kg) suggest that they had just reached puberty; and (3) the reduced number of animals on test in Part II resulted in a different loading pattern for the inhalation chamber compared to Part I. In addition, mean litter sizes were different between Parts I and II. The mean litter sizes were 9.0 fetuses/litter in the 0 ppm group in Part I versus 6.6 fetuses/litter in the 80 ppm group in Part II. Since litter size may affect fetal weight (Romero *et al.*, 1992; Duncan, 1969), it would not be appropriate to compare results from the two Parts for fetal weight comparisons.

Therefore, the results from both Parts I and II were taken into consideration in the determination of the NOEL and establishment of a NOEL at 40 ppm.

IV. CONCLUSION

DPR evaluated toxicity studies submitted and those reported in the literature and determined that methyl bromide caused developmental effects in animals. The Breslin *et al.* study (1990b) was selected as the definitive study for the establishment of a NOEL for acute exposure because the study was conducted under USEPA Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) guidelines, and the results showed statistical significance for the increased incidences of gall bladder agenesis and fused sternebrae and for the decreased fetal weight. DPR considered these developmental effects to be treatment-related and biologically significant. These effects can not be dismissed as being due to maternal toxicity since they were observed in does that did not exhibit neurotoxicity or other evidence of maternal toxicity. In addition, these effects observed in the 80 ppm treated groups were statistically significant when compared to the concurrent control group, the most appropriate group for comparison. Historical control data from the conducting laboratory, WIL Research Laboratories, MARTA (Lang, 1993), and Stadler *et al.* (1983) also supported the conclusion that the effects were treatment-related.

Further evidence of developmental toxicity can be found in the rat developmental toxicity study (Sikov *et al.*, 1981) wherein no maternal toxicity was evident. Delayed skull ossification was observed at equal litter incidence in both groups exposed to a concentration of methyl bromide at less than a maximum tolerated dose.

Methyl bromide also caused reproductive toxicity in rats. In the rat reproductive toxicity study, there was a reduction in the fertility index of the treated groups. There was also decreased pup body weights during the lactational periods for each of the four birthing periods in pups from methyl bromide treated dams. It is important to note that the pups were only exposed to methyl bromide in utero. Moreover, the in utero exposure was not continuous; that is, they were exposed to methyl bromide only 5 days per week (for a total of 15 days) instead of the daily exposure during the selected period of gestation as in the developmental toxicity protocol.

It has been suggested that developmental effects should be observed in more than one species to be confirmative. To the contrary, it is known that for some chemicals, there is species specificity in developmental effects. Developmental toxicity testing under the FIFRA guidelines requires two species to be tested, a rodent and a non-rodent species, typically the rabbit, for the purpose of identifying species susceptibility. The need to test non-rodent species arose from the findings of thalidomide where it was demonstrated that this unequivocal human teratogen did not exhibit significant teratological effects in rats but caused at least some significant effects in rabbits (Schardein, 1985a). In the absence of human data and when animal data are used, it is assumed that the most sensitive species is appropriate for use to determine regulatory action (USEPA, 1991).

Consideration should also be given to the results from genotoxicity studies which have shown that methyl bromide is an alkylating agent capable of reacting with biological nucleophiles (proteins and DNA) and is a direct-acting mutagen in a variety of test systems. While the mechanism for the developmental effects observed from the exposure to methyl bromide is unknown, it has been shown that many of the anticancer alkylating agents are teratogenic in laboratory species, and several of them have elicited malformations in humans (Schardein, 1985b). In addition, methyl chloride, the chloro analog of methyl bromide, has been shown to cause developmental effects in mice.

DPR evaluated available toxicity studies and found that methyl bromide caused developmental effects in rabbits and rats, and reproductive effects in rats. Therefore, DPR

concluded that the developmental and reproductive effects observed in laboratory animals were significant and warranted regulation on the use of methyl bromide to decrease human exposure.

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Table 1. The NOELs and LOELs of methyl bromide by inhalation from developmental and reproductive toxicity studies.

Studies	Species	Duration	ppm	NOEL/LOEL[a]	Effects
1. American Biogenics Corp. (1986)	Rat	6h/d	3 /30 3 /30		maternal-reduced fertility fetal-reduced body weights
2. Sikov *et al.*, (1981)	Rat	7h/d	70/ - 20/ 70		maternal-no significant effects fetal-delayed skull ossification
3. Sikov *et al.*, (1981)	Rabbit	7h/d	20/ 70		maternal-convulsion, paresis, and death (after 1 week)
4. Breslin *et al.*, (1990a,probe, Part I)	Rabbit	6h/d		50/-	maternal and fetal- no effects at the highest dose studied
5. Breslin *et al.*, (1990a,probe, Part II)	Rabbit lesions	6h/d		70/140	maternal- neurotoxicity (after 8 exposures), brain
6. Breslin *et al.*, (1990b,Part I)	Rabbit	6h/d	 40/ 80	40/ 80	maternal-neurotoxicity in some does (after 12 exposures) fetal-fused sternebrae, gall bladder agenesis, and other effects
7. Breslin *et al.*, (1990b,Part II)	Rabbit	6h/d	- / 80 - / 80	- / 80	maternal-decreased body weight gain fetal-gall bladder agenesis and decreased body weights

[a/] See text for full explanation of the results.

Table 2. The incidence of delayed ossification of the supraoccipital plate in rat fetuses after inhalation exposure to methyl bromide during gestation[a].

Exposure conditions[b] Premating-Gestation	Affected litters/Total litters
air - air	1/37
air - 20 ppm	2/31
air - 70 ppm	7/36[c]
20 ppm - air	4/34
20 ppm - 20 ppm	2/38
70 ppm - air	0/36
70 ppm - 70 ppm	7/36[c]

a/ Data were from Sikov *et al.*, 1981.
b/ There were 2 exposure periods: 3 weeks prior to mating, and during gestation. The does were exposed to various combinations of air or methyl bromide concentrations during those periods.
c/ p= 0.025 using Fisher's Exact Test.

Table 3. The body weights of rat pups after inhalation exposure in a 2-generation reproductive study[a].

Body Weight (grams)[b]

Lactation	F1a litter				F1b litter			
Days	0	3	30	90 ppm	0	3	30	90 ppm
0	6.0	6.2**	6.2**	6.0	6.2	6.4**	6.2	6.5**
4	9.5	9.4	9.3	9.3	9.3	9.9**	9.5	9.7*
7	13.5	13.7	13.0*	13.1	13.7	14.9**	14.1	14.3
14	23.2	22.9	21.5**	21.6**	24.1	24.2	22.5*	22.5*
21	37.8	37.7	34.3**	33.8**	39.3	39.4	36.0**	36.4**
28	68.4	66.9	62.1**	61.8**	70.1	69.3	64.1**	66.4

Lactation	F2a litter				F2b litter			
Days	0	3	30	90 ppm	0	3	30	90 ppm
0	5.6	6.1**	5.5	5.4**	6.4	6.7**	6.2	6.2
4	8.1	8.4	7.8	7.4**	10.1	9.9	9.2**	9.2**
7	11.6	12.2*	11.6	10.6**	14.3	14.7	13.4*	13.3*
14	21.9	22.6	20.4**	18.6**	24.1	23.7	19.8**	19.6**
21	35.4	36.2	31.4**	29.1**	40.3	39.8	32.4**	32.0**
28	64.3	64.2	58.6**	53.8**	71.6	70.6	58.4**	58.2**

a/ Data were from American Biogenics Corp., 1986. Fetuses were exposed in utero to methyl bromide for 5 days/week during gestation day 0 to gestation day 19. Offspring was not placed in the inhalation chambers during the lactation period.

b/ Values were mean body weights for both sexes. Statistical significance levels were * at $p \leq 0.05$, and ** at $p \leq 0.01$ levels using ANOVA and Scheffe's Multiple comparisons reported by the investigators.

Table 4. The incidence of fetal effects in rabbits after inhalation exposure to methyl bromide during gestation[a].

	Concentration (ppm)						
	Part I				Part II		
Effects	0	20	40	80	0	0[b]	80
# Examined:							
fetuses	190	137	143	159	114	102	92
litters	21	15	19	19	16	13	14
Mean litter size	9.0	9.1	7.5	8.4	7.1	7.8	6.6
Mean fetal body weight (g)	31.8	32.2	35.0	30.4	36.2	33.8	31.4*
External Effects							
omphalocele	0	0	0	2/2 (11%)[d]	0	0	0
hemorrhage (with or without generalized edema)	0	0	0	2/2 (11%)[d]	0	0	1/1 (7%)
Soft Tissues							
retroesophageal right subclavian artery	0	0	0	2/2 (11%)[d]	0	0	0
gall bladder agenesis	2/1 (5%)	1/1 (7%)	1/1 (5%)	13/5*[c] (26%)[d]	1/1 (6%)	0	4/4[c] (29%)[d]
Skeletal Effects							
fused sternebrae	0	0	3/2 (11%)	20/10*[e] (53%)[d]	NA[f]	NA[f]	NA[f]

a/ Incidence data were expressed as the number of fetuses affected/number of litters affected. Data were from Breslin, *et al.* (1990b) with does exposed to methyl bromide 6 hours/day on days 7 to 19 of gestation. Parts I and II were two separate experiments. Statistical significance in comparison to the controls, * ($p \leq 0.05$), is indicated after each incidence.

b/ These rabbits were not placed in an inhalation chamber. All does in this group had been inseminated with semen from one male that was suspected from Part I of selectively contributing to the increased incidences of gall bladder agenesis.

c/ Of the 13 fetuses with missing gall bladder in Part I, 6 were from 3 does without neurotoxicity and 7 were from 2 does with neurotoxicity. In part II, all 4 affected fetuses were from 4 does without neurotoxicity.

d/ Percent of litters affected = (affected litters/total litters examined) x 100.

e/ Of the 20 fetuses with fused sternebrae, 19 were from 9 does without neurotoxicity, and 1 from 1 doe with neurotoxicity.

f/ NA=not analyzed, i.e., skeletal examinations were not performed.

Table 5. Historical control data for developmental effects in rabbits for Dow Chemical Company 1974-1991.[a]

Effects	Fetuses[b]	Litters[c]
omphalocele	5/8956 (0.06)[d]	5/1159 (0.43)[d]
petechial hemorrhage	1/8956 (0.01)	1/1159 (0.09)
subdermal hematoma	2/8956 (0.02)	2/1159 (0.17)
generalized edema	1/8956 (0.01)	1/1159 (0.09)
retroesophageal right subclavian artery	7/5333 (0.13)	7/1158 (0.60)
gall bladder agenesis	5/5333 (0.09)	4/1158 (0.35)
fused sternebrae	55/8502 (0.65)	49/1099 (4.46)

a/ Data were from Breslin *et al.*, 1990b.
b/ Number of affected fetuses/total number of fetuses.
c/ Number of affected litters/total number of litters.
d/ Percent of total number of fetuses or litters in database affected is in parenthesis.

Table 6. Historical control data for developmental effects in rabbits for WIL Research Laboratories 1982-1992 (except as noted otherwise)[a]

Effects[b]	Fetuses[c]	Litters[d]
gall bladder agenesis	7/7232 (0.10)	7/1051 (0.67)[e]
fused sternebrae[f]	21/7855 (0.27)	18/1136 (1.58)
omphalocele	11/7855 (0.14)	11/1136 (0.97)
edema	1/7855 (0.01)[g]	1/1136 (0.09)[g]

a/ Data were from WIL Research Laboratories supplied to DPR (Holson, 1993a, 1993b, 1993c).

b/ The WIL database does not contain specific entries for hemorrhage/subdermal hematoma or retroesophageal right subclavian artery. The latter may be included under entries in which several separate blood vessel findings are grouped.

c/ Number of affected fetuses/total number of fetuses examined. Percent of incidence is in parenthesis.

d/ Number of affected litters/total number of litters examined. Percent of incidence is in parenthesis.

e/ Values are based on negative control groups from 37 studies done between February, 1985 and November, 1993.

f/ Fused sternebrae is classified as a skeletal malformation in the WIL database.

g/ Values are for localized fetal edema. An entry for generalized edema, hydrops, or words to that effect does not appear in the database.

Table 7. Historical control data for New Zealand white rabbits from the Middle Atlantic Reproduction and Teratology Association (MARTA).[a]

Effects	Fetal % Incidences Average	Litter % Incidences Average	Number of Studies	Total Litters	Total Fetuses
Gall bladder agenesis	0.14	0.92	178	2890	19310
Fused sternebrae	0.92	4.58	172	2794	18762
Omphalocele	0.07	0.32	225	2776	20071
Retroesphageal right subclavian	0.055	0.23	178	2890	19310
Hematoma	0.01	0.07	225	2776	20071
Local edema	0.02	0.18	225	2776	20071

[a/] Data were from Lang, 1993. The historical control data were compiled from 21 companies as provided by members of MARTA. Since all participating laboratories conduct studies under Good Laboratory Practices, it was assumed that conditions meet or exceed federal regulations for the care and housing of laboratory animals. Dow Chemical Company and WIL Research Laboratories were not listed as participants. In this table, only the arithmetic means of the percentage of incidences from all studies are included. These studies included rabbits which were sacrificed on gestation days 28, 29, and 30. Incidence data for effects observed in each of the gestation days are also available.

Table 8. Historical control data of New Zealand rabbits from Stadler *et al.* (1983)[a]

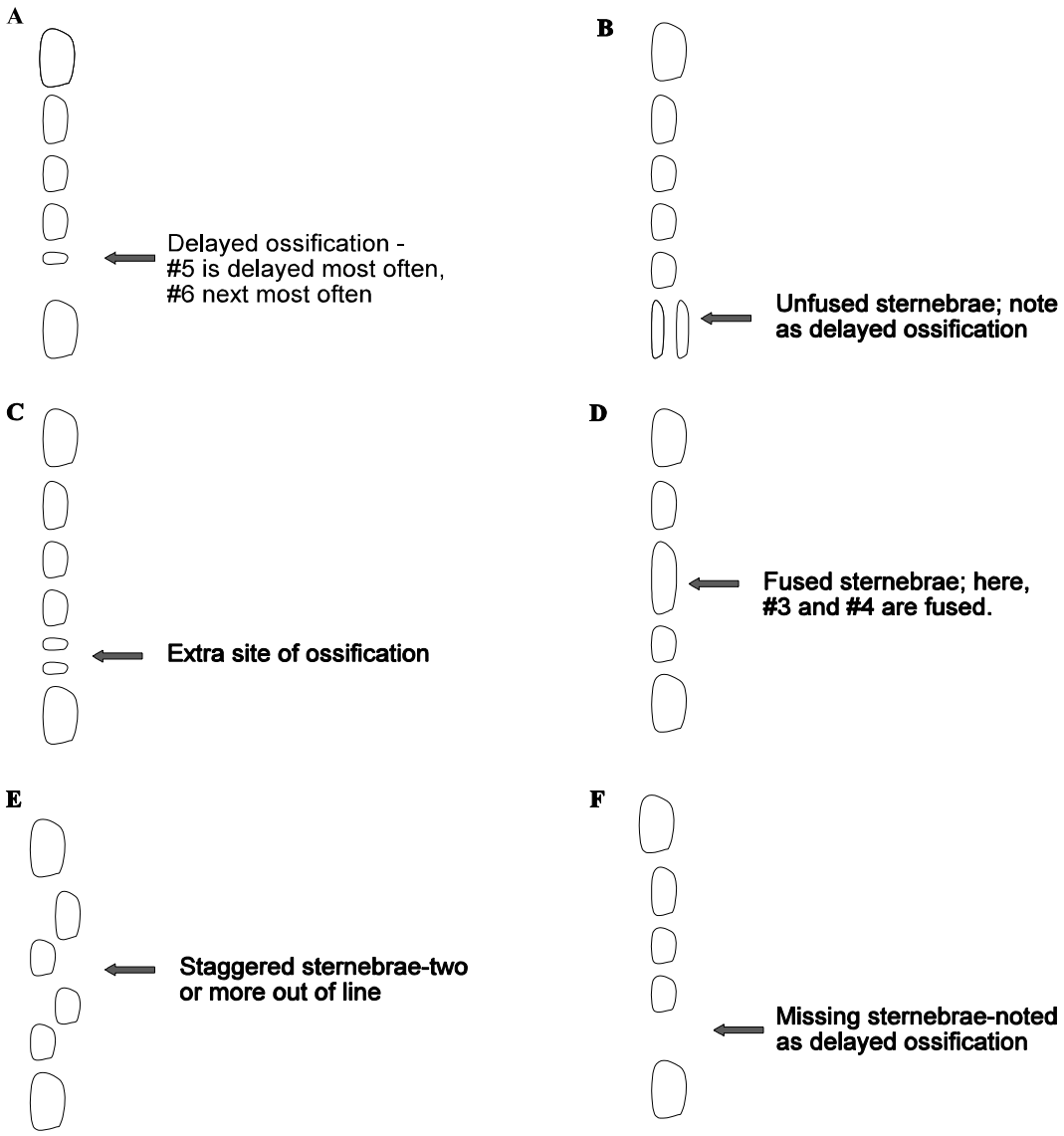
Effects[b]	Fetuses[c]
Gall bladder agenesis	6/3185 (0.19%)
Subcutaneous edema	1/5592 (0.02%)

a/ Data were from Stadler *et al.* (1983).

b/ The following endpoints did not have entries in this database: omphalocele, hemorrhaging (visible externally), retroesophageal right subclavian artery, and fused sternebrae.

c/ Values are expressed as number of fetuses affected/ total number of fetuses examined. Percent affected is included in the parenthesis. Litter incidence data were not provided in this publication.

Figure 1. Common anomalies of the sternebrae.[a]



a/ Adapted from a figure provided by The Dow Chemical Company upon request from DPR.